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Abstract: Chemical methods allowing a single soil extraction followed by multi-elemental simultaneous measurement by ICP-OES are increasingly used to predict plant uptake; however, calibration results against crop response are scarce and contradictory. Our aims were to evaluate the efficacy of five extractants to predict nutrient uptake by a greenhouse wheat crop, as well as the influence of soil properties on nutrient concentrations in soil extracts and wheat plants. Unlike other calibration studies, we monitored the pre-seeding to post-harvesting changes in soil available Ca, K, Mg, Cu, Fe, Mn, Zn and Al. We extracted 14 acidic soils (C content: 47-114 g kg^{-1}) with two traditional (AA: ammonium acetate; DTPA: diethylenetriamine-pentaacetic acid) and three multi-element extractants (AB-DTPA: ammonium bicarbonate-DTPA; Mehlich-3; AA-DTPA: ammonium acetate-DTPA). Relationships between bioavailable and chemically extractable elements were strong for K (R^2=0.776 to R^2=0.882; p<0.001) and Zn (R^2=0.663 to R^2=0.721; p<0.001), especially for AB-DTPA and AA-DTPA. Multiple regressions including also soil properties can predict wheat-Ca ($Fe_{oxihydroxides}$, clay and $Ca_{AB\text{-}DTPA}$; R^2=0.656; p<0.001) and wheat-Cu [$Al_{oxihydroxides}$ and either $Cu_{AB\text{-}DTPA}$ (R^2=0.515; p<0.01) or $Cu_{AA\text{-}DTPA}$ (R^2=0.472; p<0.01)]. Pre-seeding to post-harvesting changes in $K_{AA\text{-}DTPA}$ and $K_{AB\text{-}DTPA}$ were strongly related with K uptake by wheat (R^2=0.927 and R^2=0.949, respectively; p<0.001); similarly, for wheat-Zn the best relationships were with $Zn_{Mehlich\text{-}3}$ and $Zn_{AA\text{-}DTPA}$ (R^2=0.654 and R^2=0.757, respectively; p<0.001). Consequently, chemical extractants alone can adequately predict K and Zn bio-availability, and combined with some soil properties can predict wheat uptake of Ca and Cu, but not that of other nutrients.

1. INTRODUCTION

Soil nutrients have different and complex distribution patterns because they may be associated with the exchange phase, chelated with or contained in SOM, adsorbed or fixed on clays, adsorbed on or occluded in oxide minerals and carbonates, or be constituents of residual primary minerals (*Viets*, 1962; *Shuman*, 1991). Each of these chemical species or solid phases differs in solubility and availability to plants (*Chen et al.*, 1996), which assimilate the nutrients and trace elements as ions, acids or chelates from the soil solution (*Kirkby*, 2012). Most part of soil nutrients associated with the solid phase is not bioavailable and less than 10 % is in soluble or exchangeable forms (*Lake et al.*, 1984). Consequently, the soil total nutrient contents are not a useful index of plant available nutrients, which are assessed by chemical extractions aiming to estimate reliably the potential of soils to supply sufficient nutrients for plant growth and to retain toxic metals (*Adriano*, 2001).

Ideally, a chemical solution for assessing soil nutrient availability should extract nutrients from the same pool (*O'Connor*, 1988) and in a similar way than plants in the rhizosphere (*Alloway and Jackson*, 1991). As these conditions likely depend on the physical and chemical properties of each element, the difficulties for simulating plant uptake will increase with the number and diversity of elements considered. Furthermore, the nutrient concentration solubilized depend on the extractant characteristics (pH, neutral salts, chelating agents, acids), sample preparation (drying, grinding), extraction conditions (time, soil:extractant ratio, temperature, type of extraction vessel, shaker, shaker speed) and the soil properties *(Pickering, 1986; Sims and Johnson, 1991; Benton-Jones, 2001)*.

Chemical methods for soil available nutrient extractions can be single (*Bray and Kurtz*, 1945; *Olsen et al.*, 1954; *Lindsay and Norvell*, 1978) and multi-element or universal extractants (*Soltanpour and Schwab*, 1977; *Mehlich*, 1984; *Houba et al.*, 2000). The former are conventional and widely accepted soil tests [Bray 1, Mehlich 1 and Olsen for P; ammonium acetate (AA), for alkaline and alkaline-earth elements; diethylenetriaminepentaacetic acid (DTPA) for micro-nutrients] that, in the past, were calibrated against crop yield response. Multi-element methods [EDTA-AA, $CaCl_2$, Mehlich-3, ammonium bicarbonate-DTPA (AB-DTPA)] are increasingly employed in laboratories, because they allow a single extraction followed by simultaneous measurement of many elements by ICP-OES and it has been shown that the amounts of macro- and micro-nutrients they extract are closely related to those determined by widely accepted soil tests (*Lucena and Bascones*, 1993; *Monterroso et al.*, 1999; *Elrashidi et al.*, 2003; *Ziadi and Sen-Tran*, 2007; *Madurapperuma and Kumaragamage*, 2008; *Rodríguez-Suárez et al.*, 2008; *Sharma et al.*, 2018). However, their calibrations against crop response are still scarce and showed inconsistent results because

the amount of elements chemically extracted often does not correlate with the plant elemental content, or contrasting results were found with different crops and soils (*McLaughlin et al.*, 2000; *McBride et al.*, 2003; *Menzies et al.*, 2007; *Obrador et al.*, 2007; *da Fonseca et al.*, 2010; *Hosseinpur and Zarenia*, 2012). *McBride et al.* (2003) and *Menzies et al.* (2007) found that trace metal extracted with complexing agents (DTPA and EDTA) were poorly correlated with plant uptake, whereas neutral salts (AA, $CaCl_2$, etc.) were preferable to predict metal bioavailability over a wide range of soils. Similarly, DTPA, Mehlich-3 or AA extractants were ineffective to predict micronutrient availability for cereals in acidic soils (*Obrador et al.*, 2007; *da Fonseca et al.*, 2010), with the relative exception of Mehlich-3 and DTPA for barley-Zn (*Obrador et al.*, 2007). In acidic soils, AB-DTPA extractable K, Cu, Fe, Mn and Zn (but not Ca and Mg) are highly correlated with grass, rice and maize uptake (*Madurapperuma and Kumaragamage*, 2008; *Malathi and Stalin*, 2018), while P, S, K and Zn extracted by Mehlich-3 are correlated with biomass yield, nutrients concentration and rice uptake (*Seth et al.*, 2018).

Therefore, unfortunately, no method is universally applicable to determine bioavailable macro- and micro-nutrients (*Sauve et al.*, 2000; *Obrador et al.*, 2007) and, as highlighted *Benton-Jones* (2003), the challenge is to choose one that meets several criteria: a) multi-element extraction to take full advantage of ICP analyzers; b) suitability for a wide range of soil characteristics (pH, texture, SOM,…); and c) proved significant relationship between crop response and soil nutrients levels. According to the last criteria, extractant adequacy will increase as the difference in the amount of chemically extractable soil nutrient(s) between seeding and harvesting is closer to the amount of nutrient(s) up taken by the crop. However, to fulfil this important criteria, soil nutrient bioavailability is usually assessed by comparing crop-total nutrient concentrations with soil-extractable nutrient concentrations measured in only one moment of the crop cycle (*McLaughlin et al.*, 2000; *Chaignon et al.*, 2003; *McBride et al.*, 2003; *Menzies et al.*, 2007; *Obrador et al.*, 2007; *da Fonseca et al.*, 2010; *Soriano-Disla et al.*, 2010; *Hosseinpur and Zarenia*, 2012; *Seth et al.*, 2017). To our knowledge, the total amount of the macro- and micro-nutrients exported with a crop has not yet been compared with the seeding-to-harvesting depletion of the corresponding soil extractable pools.

Around 30 % of the ice-free world surface and 40 % of the arable land, mainly in areas where rainfall exceeds evapotranspiration, have acidic soils (*von Uexküll and Mutert*, 1995; *Kamprath and Smyth*, 2005). With $214 \cdot 10^6$ ha cropped and $734 \cdot 10^6$ tons produced worldwide, wheat is the first food crop by area harvested and the third cereal by crop production (*FAO*, 2018). Many of the major wheat growing areas have acidic soils, in which plant growth can be poor because of Al, Mn and H toxicities, as well as Ca, Mg and Mo deficiencies (*Kamprath and Smyth*, 2005; *Lollato et al.*, 2019). Consequently, the calibration of multi-elemental chemical extractants against wheat yield response in acidic soils has high agronomical and environmental importance since it would allow the optimization of the fertilizer dose on the basis of soil nutrient analysis.

We compare, by the first time, the macro- and micro-nutrient uptake by a greenhouse wheat crop with the amounts of soil nutrients chemically extracted before seeding and after harvesting. The study was done on 14 acidic soils with medium to high SOM content employing: (i) the traditional soil tests for macro- (AA) and micro-nutrients (DTPA); and (ii) the multi-element soil extractants AB-DTPA, Mehlich-3 and AA-DTPA (a combination of AA and DTPA traditional soil tests). Moreover, to improve our understanding on soil nutrient availability, the relationships among the wheat nutrient concentrations, the soil extractable nutrient concentrations before seeding and the main soil properties were also studied.

2. MATERIALS AND METHODS

2.1. Soil samples

We used 14 out of the 28 soils from the Páramo de Gavidia (Northern Andes, Venezuela) characterized by *Abadín et al.* (2002). All soils are stony, sandy and acidic Umbric Humustepts developed over post-glacial colluvia of schists and gneisses. The variability of the main soil properties was relatively tight for pH_{H2O} (4.70-5.60), moderate for sand, silt, WHC and $Fe_{oxihydroxides}$ (536-811, 94-169 g kg^{-1} dw, 298-572 and 16-33 g kg^{-1} dw, respectively), and high for total C, total N, $Al_{oxihydroxides}$ and clay (46-114, 2.5-5.5, 15-40 and 96-296 g kg^{-1} dw, respectively) (*Abadín et al.*, 2002). Each soil sample was made up of 15 sub-samples (0-15 cm depth), taken at random from the whole area of each plot with a stainless steel probe (3.5 cm in diameter), mixed, sieved (4 mm), thoroughly homogenized, air-dried and kept for analyses.

2.2. Greenhouse experiment

Soil samples (500 g) were placed in plastic pots (600 cm^3) in which 20 wheat *(Triticum aestivum* var. Callobre*)* seeds per pot (spaced about 3 cm) were sown and grown under natural light during 8 weeks. Deionized water was daily added keeping the WHC around 75 % (w/w). Wheat was chosen because its importance worldwide and because in the most usual crop-fallow chronosequence in the study area, after cropping potato for 1-2 years and before abandoning the plot, a cereal (barley or wheat) is cropped without fertilization; therefore, our wheat crop reflects perfectly the final part of the cultivation phase in this rotation.

2.3. Plant and soil analysis

The whole wheat plants were washed thoroughly with deionized water, dried to constant weight at 60 ^oC, ground (< 100 μm) and then 250 mg were digested with 2.5 ml H_2SO_4 96 % and 5 ml HNO_3 53 % in a high performance microwave (Milestone 1200 Mega, Sorisole, Italy). Total Al, Ca, Cu, Fe, K, Mg, Mn and Zn contents were analysed by ICP-OES (Varian Vista Pro, Mulgrave, Australia). Thirty seeds (on duplicate) were also treated and analysed similarly and their contribution to plant nutrient contents was subtracted in all reported data.

Available nutrients were determined in air-dried soils, before and after wheat crop, with five extractants: a) the analytical methods officially recommended in Spain to determine macro- (AA) and micro-nutrients (DTPA) separately; b) two multi-element extractants (AB-DTPA, Mehlich-3) likely to be adopted in many countries (*Hedley*, 2008) that can be used in acidic soils (*Lucena and Bascones*, 1993; *Rodríguez-Suárez et al.*, 2008); and c) a multi-element extractant which combines AA and DTPA extractants. The procedures used were as follows:

1) 1 *M* AA buffered at pH 7.0 for the analysis of macro-nutrients (*Page et al.*, 1982). The soil:extractant solution ratio was 1:10 (w/v) and the shaking time was modified from 10 to 30 min. The NH_4^+ saturates the negative charges of clay and SOM, displacing the exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, Na^+) and providing a measure of soil nutrients in the soluble and exchangeable fractions.

2) DTPA micro-nutrients soil test (*Lindsay and Norvell*, 1978): 0.005 *M* DTPA, 0.01 *M* $CaCl_2$ and 0.1 *M* triethanolamine buffered at pH 7.3. The soil:extractant solution ratio was 1:2 (w/v) and the shaking time 2 h. The DTPA chelating agent provides the most suitable combination of stability constants of the complexes with Fe, Mn Cu and Zn, allowing their extraction. As DTPA forms water-soluble micronutrient complexes, micronutrients from the most labile pools desorb from soil surfaces to replenish soil solution. The buffered pH of the extractant and its content in soluble Ca^{2+} avoid the release of $CaCO_3$-occluded unavailable micronutrients in calcareous soils, without limiting its effectiveness in acidic soils (*Sims and Johnson*, 1991).

3) AB-DTPA multi-element extractant (*Soltanpour and Schwab*, 1977): 1 *M* NH_4HCO_3 and 0.005 *M* DTPA buffered at pH 7.6. The soil:extractant solution ratio was 1:2 (w/v) and shaking time 15 min. The chemical basis for using NH_4^+ and DTPA have been previously explained for AA and DTPA soil tests. Excessive dissolution of $CaCO_3$ (that would release the occluded Cu and Zn, i.e. unavailable to plants) is prevented by including soluble HCO_3^- and by buffering the solution. Furthermore, HCO_3^- is used for the extraction of P, precipitating Ca from labile calcium phosphates as calcium carbonate, thus bringing labile P into solution. Originally developed for calcareous soils, this extractant can also be used in acidic and basic soils, with high or low SOM content (*Lucena and Bascones*, 1993).

4) Mehlich-3 multi-element extractant (*Mehlich*, 1984) at pH 2.5, used for acidic to neutral soils: 0.2 *M* CH_3COOH, 0.25 *M* NH_4NO_3, 0.015 *M* NH_4F, 0.013 *M* HNO_3 and 0.001 *M* EDTA. The soil:extractant solution ratio was 1:10 (w/v); the shaking time was increased from 5 to 15 minutes to improve method reproducibility. Acetic acid buffers the solution below pH 2.9 and, as CH_3COOH and NH_4F, allows to estimate P availability by increasing the solubility of Fe and Al phosphates and by complexing Al^{3+} that potentially bind with P. The chelating agent EDTA is included to extract the available micronutrients while NH_4^+ exchanges with basic cations (K, Ca, Mg, Na)(*Ziadi and Sen-Tran*, 2007). Although originally developed for acidic soils, it has been successfully used in a wide range of soils including calcareous soils (*Wang et al.*, 2004).

5) AA-DTPA: a mixture of 1 *M* ammonium acetate and 0.005 *M* DTPA at pH 7.0; multi-element extractant developed by the authors by combining the traditional soil tests AA and DTPA. The soil:extractant solution ratio was 1:5 (w/v) and the shaking time 2 h.

All extractions were done in a rotatory shaker and in duplicate for each soil. Extracts were filtered through 1242 Filter-lab paper and then analysed for available macro-nutrients (Ca, K and Mg), micro-nutrients (Cu, Fe, Mn and Zn) and Al by ICP-OES.

Analytical grade chemicals were used (Merck Chemical Co. and Scharlau Chemie S.A.) and all aqueous solutions were prepared with type I water (electrical conductivity max: 0.056 μS cm^{-1} at 25 ^{o}C; electrical resistivity min: 18.0 MΩ cm at 25 ^{o}C; total organic C max: 5 μg l^{-1}; Na max: 1 μg l^{-1}; Cl^- max: 1 μg l^{-1}; total Si max: 3 μg l^{-1}).

2.4. Data analysis

Correlation and simple and stepwise multiple linear regression analyses were done with SPSS 24.0 to study the relationships between: 1) the concentrations of nutrients in the wheat crop and those in the (pre-seeding) soil extracts with the five solutions, and the role of main soil properties on these relationships; and 2) the nutrients solubilized by the multi-element extracting solutions (AB-DTPA, Mehlich-3 and AA-DTPA) and the traditional soil extractants for the analysis of macro- (AA) and micro-nutrients (DTPA). After checking the fulfilment of the assumptions of linearity, independence, homocedasticity, normality and no multicollinearity, best parsimonious multiple linear regressions were selected maximizing the adjusted R^2 and minimizing the standard error of the estimated residues.

3. RESULTS

3.1. Soil extractable-nutrient content

The Mehlich-3, AB-DTPA and AA-DTPA solutions showed the highest extractant ability for soil Al, Fe and Mn, respectively, while AB-DTPA stands out by its less extractant ability for Ca and Mg (Fig. 1). The amounts of Al extracted by Mehlich-3, AB-DTPA and AA-DTPA were 5- to 100-fold higher than those extracted by AA (Table 1). Extractable macro-nutrient concentrations decreased as follows: AA-DTPA ≈ AA > Mehlich-3 >> AB-DTPA for Ca; Mehlich-3 > AA-DTPA > AB-DTPA ≈ AA for K; and AA-DTPA >> AA ≈ Mehlich-3 >>> AB-DTPA for Mg (Table 1). Compared with the micro-nutrients extracted by DTPA soil test (Table 1): a) AA-DTPA always extracted significantly higher amounts (about 2x for Mn and Zn; 3x for Fe and Cu); b) AB-DTPA extracted significantly higher amounts of Fe (4x), Cu (1.8x) and Zn (1.5x); and c) Mehlich-3 solubilized similar concentrations of Mn and Zn, and significantly higher of Cu and Fe (about 2x). Although all extractants solubilized different quantities of nutrients and Al from the 14 soils, strong linear relationships (R^2= 0.709 to 0.992; p<0.001) among the amounts solubilized by the multi-element extractants and the traditional soil-tests were observed except: a) Mehlich-3 with AA for Al (R^2= 0.552; p<0.01); b) AB-DTPA with AA for Ca (R^2=0.310; p<0.05); and c) AB-DTPA with DTPA for Cu (R^2=0.623; p<0.001).

Table 1. Mean values (and ranges between brackets) of soil nutrients concentrations (mg kg^{-1}) extracted by the soils tests (AA and DTPA) and the multielement extractants (AB-DTPA, Mehlich-3 and AA-DTPA).

Element	AA		DTPA		Mehlich-3		AB-DTPA		AA-DTPA	
Al	21	(5-72)	-	-	2019	(1587-2420)	109	(4-537)	645	(352-832)
Ca	765	(78-2133)	-	-	674	(84-1845)	204	(77-397)	812	(148-2164)
K	152	(46-257)	-	-	254	(70-515)	163	(58-294)	206	(68-366)
Mg	55	(10-170)	-	-	54	(12-194)	0.8	(0.1-4)	142	(30-492)
Cu	-	-	0.5	(0.3-1)	1	(0.7-2)	1	(0.9-2)	2	(1-3)
Fe	-	-	51	(11-129)	108	(47-209)	238	(61-498)	142	(33-290)
Mn	-	-	32	(6-49)	30	(7-43)	32	(5-51)	64	(11-98)
Zn	-	-	0.9	(0.2-4)	1	(0.4-4)	1	(0.2-5)	2	(0.2-9)

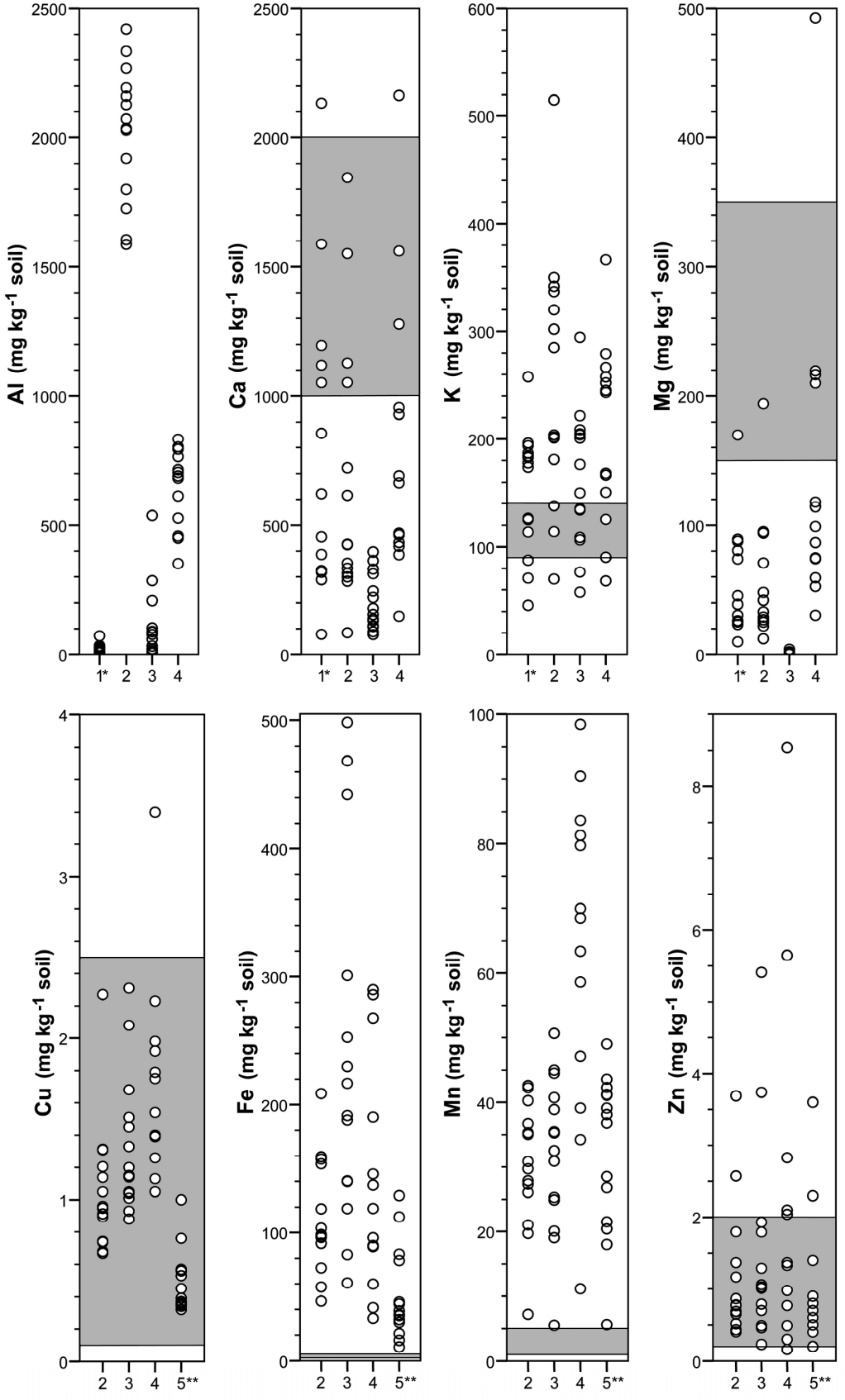

Fig. 1. Concentrations of soil-extractable macro-nutrients (Ca, K and Mg), micro-nutrients (Cu, Fe, Mn and Zn) and Al in the studied soils and range (grey shadow) of adequate concentrations in soils (following Benton Jones, 2003). Key: 1, AA; 2, Mehlich-3; 3, AB-DTPA; 4, AA-DTPA; 5, DTPA. Traditional extractant for soil macro- (*) and micro-nutrients (**).

3.2. Wheat yield and wheat nutrient content

Wheat production in the pot experiment was 8.65 ± 1.87 g kg^{-1} dry soil (mean ± s.d), ranging from 5.17 to 12.19 g kg^{-1} dry soil. Figure 2 shows the total nutrient concentrations in the aboveground biomass. Wheat yield was positively correlated (p<0.01) with the amounts of Cu (R^2= 0.368), Ca (R^2= 0.510), Zn (R^2= 0.523) and K (R^2= 0.785) taken up by the wheat plants. Contrastingly, regarding nutrients concentrations in wheat aboveground biomass, crop yield was only correlated with K richness (R^2= 0.373; p<0.001).

3.3. Relationship between wheat-total and soil-extractable nutrient content

The strongest relationships between soil-extractable (Fig. 1) and wheat-total nutrient concentrations (Fig. 2) were found for K and Zn extracted either by soil tests or multi-element solutions (R^2>0.663; p=0.001; Table 2). According to the R-values, AB-DTPA and AA-DTPA were the best extractants for K, being also slightly better than the soil test for Zn; contrastingly, Mehlich 3 was slightly better (K) and worse (Zn) than soil tests. Weaker correlations (p<0.05) with wheat-total concentrations were found for $Ca_{AB\text{-}DTPA}$ and $Fe_{AA\text{-}DTPA}$. All correlations between soil-extractable and wheat-total nutrients were positive except those for Al and Fe.

Table 2. Linear regression equations relating the wheat-total-nutrient content with the soil nutrient contents solubilized before seeding by the traditional (AA and DTPA) and the multi-element (Mehlich-3, AB-DTPA and AA-DTPA) extracting solutions.

Equation	R^2	Equation	R^2
$Al_{wheat} = 16.9 - 0.139 * Al_{AA}$	0.182	$Cu_{wheat} = 0.109 + 0.128 * Cu_{DTPA}$	0.130
$Al_{wheat} = 30.5 - 0.008 * Al_{Mehlich\text{-}3}$	0.138	$Cu_{wheat} = 0.109 + 0.060 * Cu_{Mehlich\text{-}3}$	0.127
$Al_{wheat} = 15.6 - 0.013 * Al_{AB\text{-}DTPA}$	0.115	$Cu_{wheat} = 0.139 + 0.025 * Cu_{AB\text{-}DTPA}$	0.025
$Al_{wheat} = 22.2 - 0.012 * Al_{AA\text{-}DTPA}$	0.097	$Cu_{wheat} = 0.114 + 0.033 * Cu_{AA\text{-}DTPA}$	0.077
$Ca_{wheat} = 23.6 + 0.004 * Ca_{AA}$	0.122	$Fe_{wheat} = 10.7 - 0.039 * Fe_{DTPA}$	0.129
$Ca_{wheat} = 22.9 + 0.005 * Ca_{Mehlich\text{-}3}$	0.192	$Fe_{wheat} = 11.1 - 0.022 * Fe_{Mehlich\text{-}3}$	0.068
$Ca_{wheat} = 19.8 + 0.033 * Ca_{AB\text{-}DTPA}$	0.298*	$Fe_{wheat} = 11.9 - 0.013 * Fe_{AB\text{-}DTPA}$	0.236
$Ca_{wheat} = 22.0 + 0.006 * Ca_{AA\text{-}DTPA}$	0.228	$Fe_{wheat} = 12.4 - 0.023 * Fe_{AA\text{-}DTPA}$	0.306*
$K_{wheat} = 6.68 + 0.607 * K_{AA}$	0.776***	$Mn_{wheat} = 2.36 + 0.028 * Mn_{DTPA}$	0.054
$K_{wheat} = 21.6 + 0.303 * K_{Mehlich\text{-}3}$	0.788***	$Mn_{wheat} = 2.17 + 0.036 * Mn_{Mehlich\text{-}3}$	0.057
$K_{wheat} = 3.79 + 0.583 * K_{AB\text{-}DTPA}$	0.882***	$Mn_{wheat} = 2.08 + 0.037 * Mn_{AB\text{-}DTPA}$	0.092
$K_{wheat} = 8.17 + 0.450 * K_{AA\text{-}DTPA}$	0.880***	$Mn_{wheat} = 2.60 + 0.012 * Mn_{AA\text{-}DTPA}$	0.042
$Mg_{wheat} = 10.2 + 0.025 * Mg_{AA}$	0.098	$Zn_{wheat} = 0.106 + 0.099 * Zn_{DTPA}$	0.712***
$Mg_{wheat} = 10.1 + 0.028 * Mg_{Mehlich\text{-}3}$	0.163	$Zn_{wheat} = 0.080 + 0.101 * Zn_{Mehlich\text{-}3}$	0.663***
$Mg_{wheat} = 10.3 + 1.66 * Mg_{AB\text{-}DTPA}$	0.265	$Zn_{wheat} = 0.103 + 0.064 * Zn_{AB\text{-}DTPA}$	0.721***
$Mg_{wheat} = 9.84 + 0.013 * Mg_{AA\text{-}DTPA}$	0.202	$Zn_{wheat} = 0.125 + 0.039 * Zn_{AA\text{-}DTPA}$	0.714***

*** significant at p<0.001; ** significant at p<0.01; * significant at p<0.05.

The strongest relationships of the post-wheat minus pre-wheat soil-extractable nutrient concentrations with nutrient taken up by the crop were also found for K (Fig. 3) and Zn (Fig. 4), best results being obtained with $K_{AB\text{-}DTPA}$ and $K_{AA\text{-}DTPA}$ (R^2>0.927; > 92 % of the variance explained) and $Zn_{AA\text{-}DTPA}$ (R^2=0.757; 76 % of the variance explained). Usually 1-86 % of the extractable soil nutrients was taken up by wheat (Table 3) but values higher than 100 % were found for Al_{AA} (8 soils), $Al_{AB\text{-}DTPA}$ (5 soils), $Mg_{AB\text{-}DTPA}$ (14 soils) and Fe_{DTPA} (2). Therefore, those methods were unable to predict Al, Mg and Fe availability for wheat in the studied soils.

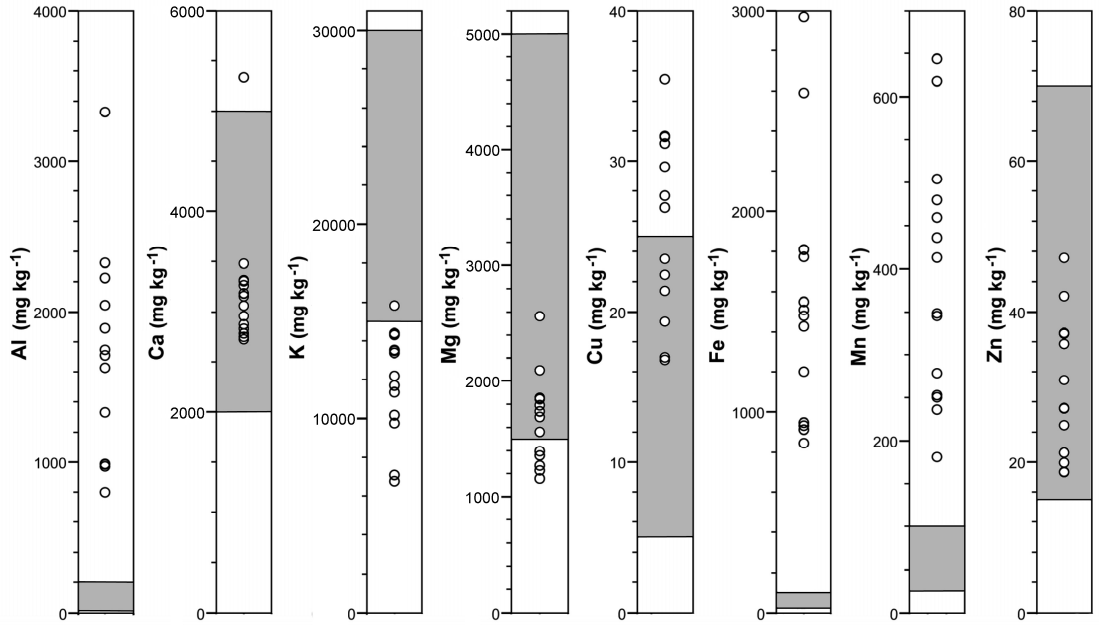

Fig. 2. Total Al, Ca, K, Mg, Cu, Fe, Mn and Zn concentrations in wheat grown in the studied soils and range (grey shadow) of adequate wheat-total-nutrient concentrations (following Benton Jones, 2003).

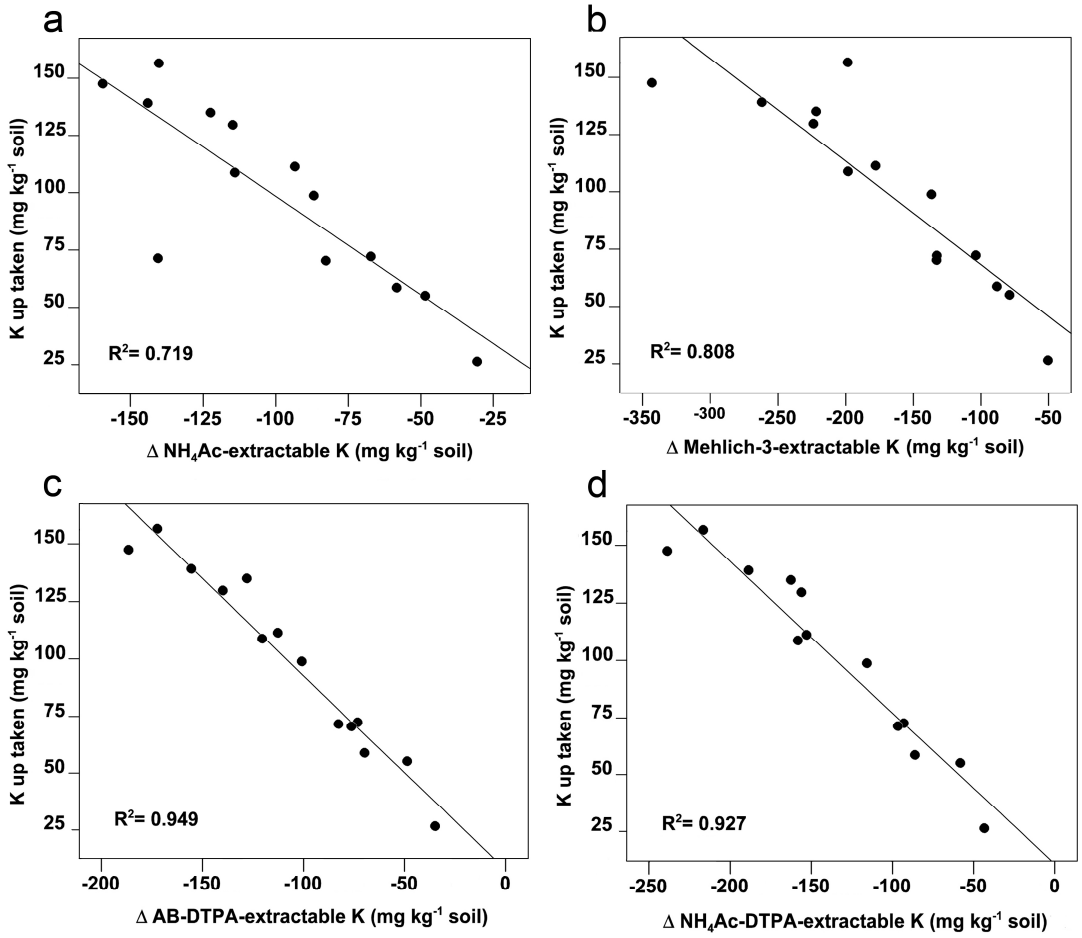

Fig. 3. Relationships of K taken up by wheat with the differences (Δ) in soil extractable K between harvest and seeding (mg kg^{-1} soil) for: a) AA; b) Mehlich-3; c) AB-DTPA; and d) AA-DTPA.

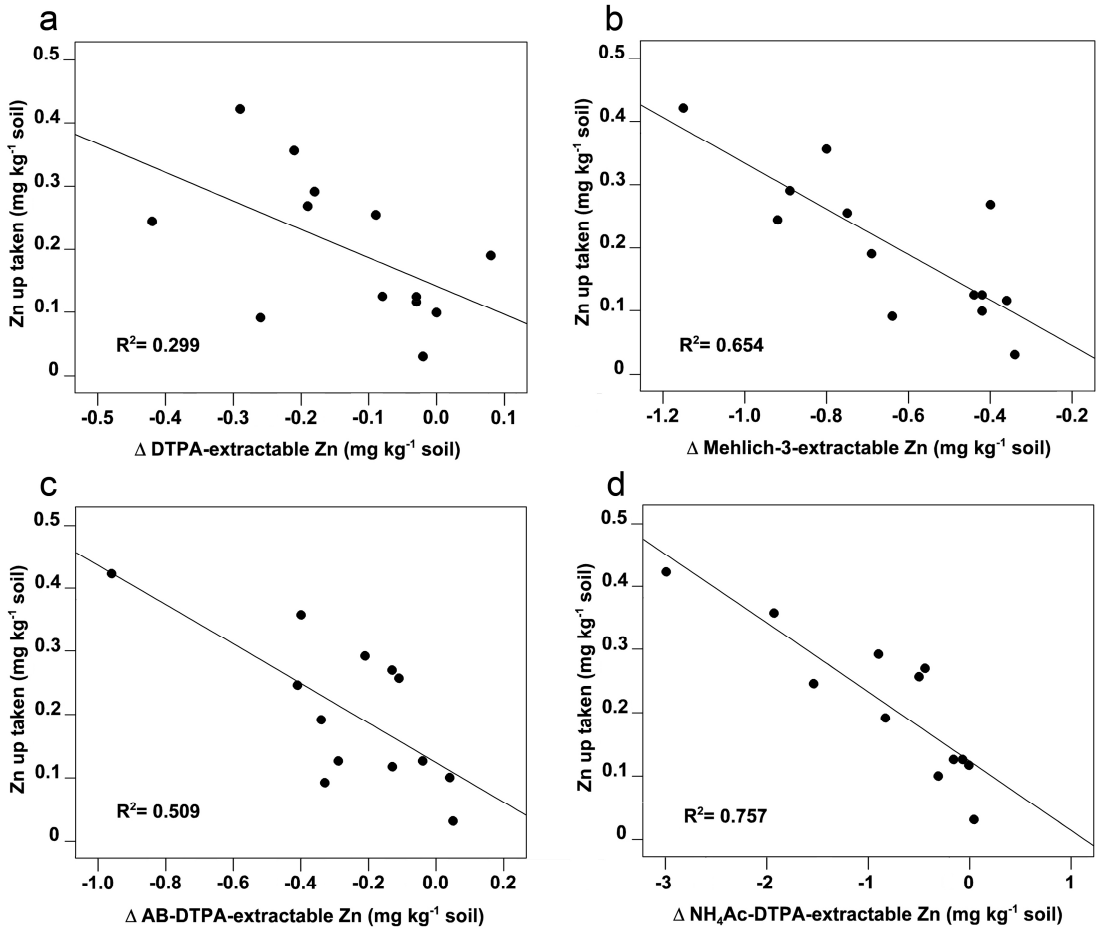

Fig. 4. Relationships of Zn taken up by wheat with the differences in soil extractable Zn between harvest and seeding (mg kg^{-1} soil) for: a) DTPA; b) Mehlich-3; c) AB-DTPA; and d) AA-DTPA.

Table 3. Mean values (and ranges between brackets) of the percentages of nutrients (and Al) chemically extracted from soil before seeding which were taken up by wheat.

Element	AA		DTPA		Mehlich-3		AB-DTPA		AA-DTPA	
Al	142	(31- 382)	-	-	1	(0.4-3)	86	(4-386)	3	(1-5)
Ca	6	(2-20)	-	-	6	(2-18)	16	(6-28)	5	(2-10)
K	66	(39-84)	-	-	40	(29-55)	60	(46-72)	49	(39-61)
Mg	32	(9-66)	-	-	31	(8-53)	5535	(400-16200)	12	(3-24)
Cu	-	-	38	(19-85)	17	(11-39)	14	(7-31)	11	(5-26)
Fe	-	-	32	(5-106)	10	(3-29)	6	(1-20)	12	(2-41)
Mn	-	-	12	(4-30)	12	(5-23)	12	(5-31)	6	(2-15)
Zn	-	-	30	(12-65)	19	(8-35)	17	(8-39)	17	(5-40)

3.4. Relationships among biologically and chemically available nutrients and soil properties

Except for K and Zn, significant and consistent correlations between wheat-total and soil-extractable nutrient concentrations were not observed. Therefore, stepwise multiple linear regressions were done for each other wheat nutrient as dependent variable and main soil properties and soil-extractable nutrients as independent variables. Three significant relationships were found:

Ca_{wheat} = 26.7 + 4.93*$Ca_{AB\text{-}DTPA}$ - 7.02*$Fe_{oxihydroxides}$ + 5.69*Clay (R^2=0.656; p<0.001)

Cu_{wheat} = 0.216 + 0.111*$Cu_{AB\text{-}DTPA}$ - 0.088*$Al_{oxihydroxides}$ (R^2=0.515; p<0.01)

Cu_{wheat} = 0.208 + 0.055*$Cu_{AA\text{-}DTPA}$ - 0.065*$Al_{oxihydroxides}$ (R^2=0.472; p<0.01)

The soil properties related with biological and chemical extraction of soil nutrients were assessed by stepwise multiple linear regressions, with either wheat-nutrient concentrations or soil-extractable nutrient concentrations as dependent variables and soil properties as independent variables (Tables 4 and 5). All best models for the concentrations of K_{wheat} and soil-extractable K (53-70 % of the variances explained) included soil pH, WHC and $Al_{oxihydroxides}$ content as independent variables. For Ca_{wheat} no valid regression was found, while soil-extractable Ca depended mostly on soil pH_{H2O}, WHC and $Al_{oxihydroxides}$ (56-83 % of the variance explained). Mg_{wheat} was weakly related with soil pH_{KCl} or texture (sand and silt contents), which explained only 22-28% of its variance, while $Mg_{Mehlich\text{-}3}$, $Mg_{AB\text{-}DTPA}$ and $Mg_{AA\text{-}DTPA}$ were related with soil pH_{H2O}, $Al_{oxihydroxides}$ and silt or WHC; no valid model was obtained for Mg_{AA}.

Table 4. Best multiple linear regressions models with Al and macro-nutrients taken up by wheat or chemically extracted from soil before seeding as dependent variables and main soil characteristics as independent variables. Notes: the name of variables in *italic* indicates that the Z scores of the variables were used.

Multiple linear regressions models	**Adjuste**
Al_{wheat} = 12.8 - 8.23*C - 3.79*$Sand$	0.368*
Al_{wheat} = 13.9 - 6.05*N - 3.69*$Sand$	0.351*
Al_{AA} = -10.1 + 11.8*Al_{oxides}	0.360*
Al_{AA} = 10.9 -2.10*Silt + 15.4*Al_{oxides}	0.491**
$Al_{Mehlich\text{-}3}$ = 2039 + 181*$Sand$ + 317*Al_{oxides}	0.618***
$Al_{AB\text{-}DTPA}$ = -109 + 81.4*Al_{oxides}	0.188
$Al_{AA\text{-}DTPA}$ = 1452- 247*pH_{H2O} + 205*Al_{oxides}	0.580**
$Al_{AA\text{-}DTPA}$ = 682 - 69.2*pH_{H2O} + 72.0*$Sand$ + 199*Al_{oxides}	0.740***
Ca_{wheat} = no valid model was found	
Ca_{AA} = 765+ 273*pH_{H2O} + 592*WHC - 714*Al_{oxides}	0.656***
$Ca_{Mehlich\text{-}3}$ = 674 + 285*pH_{H2O} + 501*N - 522*Al_{oxides}	0.830***
$Ca_{AB\text{-}DTPA}$ = -761 + 163*pH_{H2O} + 29.7*WHC - 123*Al_{oxides}	0.689***
$Ca_{AB\text{-}DTPA}$ = -2663 + 450*$pH_{p\text{-}nitro}$ + 25.4*Silt	0.564**
$Ca_{AA\text{-}DTPA}$ = 2787 + 884*pH_{H2O} - 1048*Al_{oxides}	0.777***
K_{wheat} = 98.7 + 13.2*pH_{H2O} - 23.7*pH_{KCl} + 38.3*WHC - 51.6*Al_{oxides}	0.527**
K_{AA} = 152 + 26.2*pH_{H2O} - 46.2*pH_{KCl} + 48.9*WHC - 69.8*Al_{oxides}	0.582**
$K_{Mehlich\text{-}3}$ = 254 + 59.6*pH_{H2O} - 87.5*pH_{KCl} + 116*WHC - 156*Al_{oxides}	0.701***
$K_{AB\text{-}DTPA}$ = 163 + 31.8*pH_{H2O} - 44.1*pH_{KCl} + 57.1*WHC - 84.4*Al_{oxides}	0.601**
$K_{AA\text{-}DTPA}$ = 195 + 46.9*pH_{H2O} - 72.8*pH_{KCl} + 68.8*WHC - 123*Al_{oxides}	0.639***
Mg_{wheat} = -20.7* + 7.72*pH_{KCl}	0.223
Mg_{wheat} = 11.6 + 2.97*$Sand$ + 3.18*$Silt$	0.283*
Mg_{AA} = no valid model was found	
$Mg_{Mehlich\text{-}3}$ = -553 + 106*pH_{H2O} + 4.91*WHC - 60.1*Al_{oxides}	0.464**
$Mg_{AB\text{-}DTPA}$ = -9.43 + 1.77*pH_{H2O} + 0.22*Silt - 0.84*Al_{oxides}	0.456**
$Mg_{AA\text{-}DTPA}$ = -879 + 176*pH_{H2O} + 30.1*Silt - 135*Al_{oxides}	0.425*

*** significant at p<0.001; ** significant at p<0.01; * significant at p<0.05.

The best linear regressions for Al_{wheat} concentrations included the properties related with SOM (N and C contents; R^2=0.368; p<0.05) and texture (sand content; R^2=0.351; p<0.05) as independent variables. Contrastingly, the Al chemically extracted seemed to depend most on $Al_{oxihydroxides}$ content alone (Al_{AA}: R^2=0.360, p<0.05; $A_{AB-DTPA}$: R^2=0.188, n.s.) or on soil pH_{H2O} and texture (other extractants; R^2 =0.351 to 0.740; p=0.05 to 0.001).

Table 5. Best multiple linear regressions models with micro-nutrients taken up by wheat or chemically extracted from soil before seeding as dependent variables and main soil characteristics as independent variables. Notes: the name of variables in *italic* indicates that the Z scores of the variables were used.

Multiple linear regressions models	**Adjusted R^2**
Cu_{wheat} = 0.949 - 0.008*Sand - 0.127*Al_{oxides}	0.523**
Cu_{wheat} = 0.104 + 0.007*WHC - 0.110*Al_{oxides}	0.720***
Cu_{DTPA} = -2.16 + 0.459*pH_{H2O} + 0.018*WHC	0.386*
$Cu_{Mehlich-3}$ = -2.99 + 0.791*pH_{H2O}	0.232
$Cu_{AB-DTPA}$ = -0.687 + 0.049*WHC	0.522**
$Cu_{AB-DTPA}$ = 0.241 + 0.168*C	0.641***
$Cu_{AA-DTPA}$ = 1.68 + 0.264*pH_{H2O} + 0.492*$Clay$ - 0.433*Al_{oxides}	0.508**
$Cu_{AA-DTPA}$ = 1.73 + 0.237*pH_{H2O} + 0.429*WHC - 0.539*Al_{oxides} + 0.322*Fe_{oxides}	0.655***
Fe_{wheat} = 21.2 - 32.8*N	0.555**
Fe_{DTPA} = 244 - 50.6*pH_{KCl} + 374*N - 51.0*Al_{oxides}	0.804***
Fe_{DTPA} = 8.44 + 407*N - 46.7*Al_{oxides}	0.888***
$Fe_{Mehlich-3}$ = 91.1 + 453*N - 64.4*Al_{oxides}	0.710***
$Fe_{AB-DTPA}$ = -144 + 1006*N	0.374*
$Fe_{AA-DTPA}$ = -185 + 890*N	0.613***
$Fe_{AA-DTPA}$ = -60.0 + 1069*N - 83.9*Al_{oxides}	0.831***
Mn_{wheat} = 25.2 - 4.24*pH_{H20}	0.619***
Mn_{DTPA} = 3.61 + 80.5*N	0.217
Mn_{DTPA} = -14.2 + 1.17*WHC	0.213
$Mn_{Mehlich-3}$ = 89.9 - 0.89*Sand	0.374*
$Mn_{Mehlich-3}$ = -13.6 +1.09*WHC	0.331*
$Mn_{AB-DTPA}$ = -16.5 + 7.78*C	0.661***
$Mn_{AB-DTPA}$ = -12.7 + 123*N	0.612***
$Mn_{AA-DTPA}$ = -21.3 + 13.4*C	0.470**
$Mn_{AA-DTPA}$ = -62.6 + 3.10*WHC	0.436*
Zn_{wheat} = 0.195 + 0.953*N - 0.152*Al_{oxides}	0.528**
Zn_{wheat} = 1.054 - 0.188*pH_{KCl} + 0.815*N - 0.158*Al_{oxides}	0.607**
Zn_{DTPA} = 0.929 - 0.937*$pH_{p-nitro}$ - 0.741*Al_{oxides}	0.567**
$Zn_{Mehlich-3}$ = 1.17 - 0.523*pH_{KCl} + 0.757*WHC - 0.987*Al_{oxides}	0.489**
$Zn_{AB-DTPA}$ = 13.2 - 2.89*pH_{KCl} + 9.8*N - 1.38*Al_{oxides}	0.437**
$Zn_{AA-DTPA}$ = 74.5 -12.1*$pH_{p-nitro}$ - 2.56*Al_{oxides}	0.563**

*** significant at p<0.001; ** significant at p<0.01; * significant at p<0.05.

Wheat-Mn was related with soil pH_{H2O} whereas soil-extractable Mn was mostly associated with WHC (DTPA, Mehlich-3 and AA-DTPA) and SOM-related properties, the total contents of N (DTPA and AB-DTPA) and C (AB-DTPA and AA-DTPA). The best linear regressions for Cu_{wheat} related it with

$Al_{oxihydroxides}$ and either WHC or texture (sand content) (R^2=0.523 to 0.720, p<0.05 to p<0.001), whereas Cu_{DTPA} was related with soil pH and WHC (R^2=0.386, p<0.05); $Cu_{Mehlich-3}$ with soil pH (R^2=0.232, n.s.); $Cu_{AB-DTPA}$ with WHC (R^2=0.522, p<0.01) or C (R^2=0.641, p<0.001); and $Cu_{AA-DTPA}$ with pH, $Al_{oxihydroxides}$ and either WHC (R^2=0.508, p<0.01) or clay (R^2=0.655, p<0.001). While Fe_{wheat} (R^2=0.555, p<0.01), $Fe_{AB-DTPA}$ (R^2=0.374, p<0.05) and $Fe_{AA-DTPA}$ (R^2=0.613, p=0.001) were related with N content, Fe_{DTPA} and $Fe_{Mehlich-3}$ were also related with $Al_{oxihydroxides}$ (R^2=0.888 and 0.710, respectively, p<0.001). The model for $Fe_{AA-DTPA}$ was improved when $Al_{oxihydroxides}$ content was also included (R^2=0.831, p<0.001). The best models for Zn_{wheat} (R^2=0.607, p<0.001) and $Zn_{AB-DTPA}$ (R^2=0.437, p=0.01) included N content, $Al_{oxihydroxides}$ and soil pH_{KCl} as independent variables, while WHC instead N was included in that for $Zn_{Mehlich-3}$. However, Zn_{DTPA} and $Zn_{AA-DTPA}$ were related with soil $pH_{p\text{-nitrophenol}}$ and $Al_{oxihydroxides}$ (R^2=0.567 and 0.563, respectively, p<0.01).

4. DISCUSSION

4.1. Soil extractable-nutrients

Despite their different characteristics (EDTA or DTPA chelating agents that differ in metal-complex stability; acidic to neutral or basic pH) and extraction efficacy, strong relationships existed among all nutrients extracted by the multi-element extractants (Mehlich-3, AB-DTPA and AA-DTPA) and the AA and DTPA soil tests (R^2=0.552, p<0.01, to R^2=0.992, p<0.001) in our acidic soils, the only exception being $Ca_{AB-DTPA}$. This result could be explained by the use of ammonium salts as the extracting agent for macronutrients in all methods and by the similar complexing capacity of both chelating agents (EDTA and DTPA). These correlations suggest that all methods extracted from the same (or similar) pools of solids-bound nutrients (*McBride et al.*, 2003; *Seth et al.*, 2018), as has also been reported on acidic soils around the world. Regarding the macro-nutrients, the amounts extracted with the soil test AA are strongly correlated with those solubilised with AB-DTPA (*Lucena and Bascones*, 1993; *Elrashidi et al.*, 2003; *Sharma et al.*, 2018; *Zhang et al.*, 2018), acid AA-DTPA (*Sharma et al.*, 2018), AA-EDTA (*Rodríguez-Suárez et al.*, 2008) and Mehlich-3 (*Elrashidi et al.*, 2003; *Wang et al.*, 2004; *Zhang et al.*, 2018). For the micro-nutrients, the quantities extracted with DTPA are strongly correlated with those extracted with AB-DTPA (*Elrashidi et al.*, 2003; *Behera et al.*, 2011; *Behera and Shukla*, 2014; *Sharma et al.*, 2018; *Zhang et al.*, 2018), acid AA-DTPA (*Sharma et al.*, 2018), AA-EDTA (*Rodríguez-Suárez et al.*, 2008) and Mehlich-3 (*Monterroso et al.*, 1999; *Caridad Cancela et al.*, 2002; *Elrashidi et al.*, 2003; *Wang et al.*, 2004; *Rodríguez-Suárez et al.*, 2008; *Behera et al.*, 2011; *Behera and Shukla*, 2014; *Zhang et al.*, 2018)

Our results show that Mehlich-3 extracts similar amounts of nutrients as DTPA and AA soil-tests in more cases (Ca, Mg, Mn and Zn) than the other multi-element solutions, but 50% more Fe and Cu than DTPA. In acid soils from the same region, (*Monterroso et al.*, 1999) found strong correlations between DTPA and Mehlich-3 for Fe, Cu and Zn and a higher extractant capacity for Mehlich-3. The latter result is explained by the strong acidity of Mehlich-3 (pH 2.5) that allows a higher solubilization of soil nutrients than DTPA (pH 7.3). Similar amounts (*Ziadi and Sen-Tran*, 2007) and significant correlations have also been found between the exchangeable cations displaced with Mehlich-3 and AA (*Monterroso et al.*, 1999; *Elrashidi et al.*, 2003; *Wang et al.*, 2004). Furthermore, Mehlich-3 extracted more Al than the other extractants, likely due to its acidic pH (*Monterroso et al.*, 1999).

AA-DTPA had the highest extraction efficacy for most nutrients (Ca, Mg, Cu, Mn and Zn). This result could be explained by the higher extraction time (2 h vs 15 min) and the complexing capacity of CH_3COO^- and NH_4^+ (through amine complexes) that mobilise nutrients to the soil solution (*Pueyo et al.*, 2004; *Meers et al.*, 2007). Acetate anion concentration is 5 times higher in AA-DTPA than in Mehlich-3, which also includes other anions (NO_3^- and F^-) but with less complexing capacity (*Meers et al.*, 2007). Although the three multielement extractants are NH_4^+ based solutions, Mehlich-3 has the lower concentration.

AB-DTPA stands out by its less extractant ability for Ca and Mg, probably due to the HCO_3^- in this extractant and the high levels of soluble and exchangeable soil Ca and Mg that cause their precipitation as carbonates during the extraction (*Soltanpour*, 1985).

4.2. Wheat yield and nutrient uptake

The relationships of wheat production with K uptake and concentration in the harvested biomass are easily explained considering the key role of K for crop yield (*Pettigrew*, 2008). Regarding the other nutrients related with wheat yield in our study, Ca deficiency of wheat has been reported in acidic sandy soils like ours (*Adcock et al.*, 2001), wheat is known as Cu sensitive crop (*Broadley et al.*, 2012) and Zn deficiency is frequent in cereals (*Singh et al.*, 2005). The strong correlation we found between yield and Zn uptake in wheat was previously found in maize by *Joshi et al.* (2014), who highlighted that the acid soluble and displaceable Zn fractions were the most important for plants in acidic soils.

4.3. Wheat-total and soil-extractable nutrient content

A significant relationship between soil extractable nutrients and crop response is one of the most important criteria for selecting soil extractants (*Benton-Jones*, 2003). In the last decade, biological availability and chemical extractability with multi-element extractants have been compared for many nutrients and crops (*da Fonseca et al.*, 2010; *Soriano-Disla et al.*, 2010; *Gediga et al.*, 2015; *Korzeniowska and Stanislawska-Glubiak*, 2015; *Joshi et al.*, 2017; *Seth et al.*, 2017; *Zhu et al.*, 2017; *Asensio et al.*, 2018; *Seth et al.*, 2018). However, this relationship remains unclear because it depends on plant species and soil characteristics and, moreover, soil nutrient bioavailability has been assessed by comparing crop-total and soil nutrients extracted at a single temporal point. Aiming to overcome this possible limitation, we evaluated the extractant abilities to assess soil nutrient bioavailability by comparing the pre-seeding to post-harvesting depletion of soil-extractable nutrient pools with the wheat-total nutrient uptake.

All extractants adequately predict K bioavailability, but the linear regressions of wheat-total concentrations with the pre-seeding to post-harvest changes in soil-extractable concentrations pointed to AB-DTPA and AA-DTPA as the best extractants for K. Despite the differences in soils and crops, our results agree with those of *Zhu et al.* (2017) and *Seth et al.* (2018) for Mehlich-3 and AB-DTPA. Unlike *Panda and Patra* (2018), we do not need multiple regression models including soil variables because both AB-DTPA and AA-DTPA explain >93% of the variance of K bioavailability

Both AB-DTPA and Mehlich-3 can predict Zn uptake by wheat in our soils (51% and 61% of the variance explained, respectively), as previously reported for rice and maize (*Joshi et al.*, 2014; *Seth et al.*, 2017; *Seth et al.*, 2018), but contrasting with other results for wheat and barley (*da Fonseca et al.*, 2010; *Soriano-Disla et al.*, 2010). However, the linear regressions of wheat-total concentrations with the pre-seeding to post-harvest differences of soil-extractable Zn, showed that AA-DTPA was the best extractant in our soils (76% of variance explained).

Although correlations are less significant, Ca and Fe bioavailability for wheat can still be predicted by $Ca_{AB\text{-}DTPA}$ and $Fe_{AA\text{-}DTPA}$. However, soil-extractable Fe levels have not a clear trend during the wheat growth, post-harvest concentration being higher or lower than the pre-seeding one depending on soils and extractants (data not shown). Consequently, the checked extractants shared a questionable suitability to predict Fe availability for wheat in these soils. While we did not find significant relations for other nutrients, contrasting results have been reported for the extractable amounts of Cu, Fe and Mn and crop uptake (*da Fonseca et al.*, 2010; *Gediga et al.*, 2015; *Korzeniowska and Stanislawska-Glubiak*, 2015; *Joshi et al.*, 2017).

4.4. Biological and chemical availability of nutrients and soil properties

The plant availability of nutrients is controlled by soil properties as pH, SOM, Fe, Mn and $Al_{oxyhydroxides}$, texture and moisture (*Fageria et al.*, 2002; *Seth et al.*, 2018). As only wheat K and Zn were consistently correlated with soil extractable nutrient contents, linear stepwise multiple regression analyses were done to evaluate if the prediction of plant-nutrient availability could be improved by including some soil properties, as reported other studies (*Lindsay and Cox*, 1985; *Soriano-Disla et al.*, 2010; *Bilias and Barbayiannis*, 2017; *Panda and Patra*, 2018). We did not find significant regressions for wheat Al, Mg, Fe and Mn, but two thirds of Ca_{wheat} variance was explained by a regression model with soil $Fe_{oxihydroxides}$, clay, and $Ca_{AB\text{-}DTPA}$ as independent variables. These three variables are closely related with soil Ca dynamics: a) although $Fe_{oxihydroxides}$ have low exchange capacity and their charges depend on soil pH, they are powerful adsorbents of anions and cations (*Schwertmann and Taylor*, 1989); b) soil Ca is retained as an exchangeable form at the charged silicate clay surfaces; and c) $Ca_{AB\text{-}DTPA}$ is an index of Ca availability. In the case of Cu_{wheat}, around half of its variance was explained by soil $Al_{oxihydroxides}$ and either

$Cu_{AB\text{-}DTPA}$ or $Cu_{AA\text{-}DTPA}$. Like those of Fe, $Al_{oxihydroxides}$ are powerful adsorbents of anions and cations (*Schwertmann and Taylor*, 1989) and, according to *Gupta and Aten* (1993), Cu bioavailability may be influenced by soil metal oxihydroxides, while *Chaignon et al.* (2003) reported a significant correlation of the extractable Cu with $Fe_{oxihydroxides}$. As they are closely associated with SOM content, the inclusion of $Al_{oxihydroxides}$ in the regression model for Cu_{wheat} could also be partly due to the well-known relationship between extractable Cu and SOM (*Katyal and Sharma*, 1991; *Caridad Cancela et al.*, 2002; *Chaignon et al.*, 2003).

The regression analyses with either wheat-total nutrients or soil-extractable nutrients as dependent variables showed that the biological and chemical availability were related with the same soil properties only for K (pH, WHC and $Al_{oxihydroxides}$), Fe (total N) and Zn (total N, $Al_{oxihydroxides}$ and pH). These results suggested that wheat plants take up these three nutrients from the same soil pools than the studied chemical extractants. Analogous relationships of extractable-K, Fe and Zn with soil pH or SOM related variables have been previously reported (*Barber*, 1984; *Behera et al.*, 2011; *Behera and Shukla*, 2014; *Seth et al.*, 2017).

5. CONCLUSIONS

In our sandy and acidic Umbric Humustepts studied, the assayed chemical extractants adequately predicted the wheat uptake for only two (K and Zn) out of eight elements considered, suggesting a limited utility of these chemical methods to predict in acidic soils (i.e., around one third of the world surface) the nutrition of the most widely cultivated food crop. Uptake of Ca and Cu can be also predicted when results from some extractants are combined with data on some soil properties. Being multi-element extractants and providing better results than the traditional soil tests (at least for K, Zn, Ca and Cu), AA-DTPA and AB-DTPA can be the most promising of the assayed methods for future developments. However, it must be taken into account that: a) AB-DTPA was unable to reflect the wheat uptake of Al and Mg in the studied soils; and b) like the other studied extractants, the suitability of both AA-DTPA and AB-DTPA for predicting Fe bioavailability in these soils is questionable.

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References

Abadín, J., González-Prieto, S. J., Sarmiento, L., Villar, M. C., Carballas, T. (2002): Successional dynamics of soil characteristics in a long fallow agricultural system of the high tropical Andes. *Soil Biol. Biochem.* 34(11), 1739-1748.

Adcock, K. G., Gartrell, J. W., Brennan, R. F. (2001): Calcium deficiency of wheat grown in acidic sandy soil from Southwestern Australia. *J. Plant Nutr.* 24(8), 1217-1227.

Adriano, D. C. (2001): Trace elements in terrestrial environments. Springer-Verlag, New York.

Alloway, B. J., Jackson, A. P. (1991): The behavior of heavy-metals in sewage sludge-amended soils. *Sci. Total Environ.* 100, 151-176.

Asensio, V., Abreu-Junior, C. H., da Silva, F. C., Chitolina, J. C. (2018): Evaluation of chemical extractants to assess metals phytoavailability in Brazilian municipal solid waste composts. *Environ. Pollut.* 243, 1235-1241.

Barber, S. A. (1984): Soil nutrient bioavailability. A mechanistic approach. John Wiley & Sons, New York.

Behera, S. K., Shukla, A. K. (2014): Total and Extractable Manganese and Iron in Some Cultivated Acid Soils of India: Status, Distribution and Relationship with Some Soil Properties. *Pedosphere* 24(2), 196-208.

Behera, S. K., Singh, M. V., Singh, K. N., Todwal, S. (2011): Distribution variability of total and extractable zinc in cultivated acid soils of India and their relationship with some selected soil properties. *Geoderma* 162(3-4), 242-250.

Benton-Jones, J. (2001): Laboratory Guide for Conducting Soil Tests and Plant Analysis. CRC Press, Boca Raton.

Benton-Jones, J. (2003): Agronomic Handbook. Management of crops, soils and their fertility. CRC Press LLC,

Boca Ratón. Florida.
Bilias, F., Barbayiannis, N. (2017): Evaluation of sodium tetraphenylboron (NaBPh4) as a soil test of potassium availability. *Arch. Agron. Soil Sci.* 63(4), 468-476.
Bray, R. H., Kurtz, L. T. (1945): Determination of total, organic, and available forms of phosphorus in soils. *Soil Sci.* 59(1), 39-45.
Broadley, M., Brown, P., Cakmak, I., Rengel, Z., Zhao, F. (2012): Chapter 7 - Function of Nutrients: Micronutrients, in Marschner, P. (ed.): Marschner's Mineral Nutrition of Higher Plants (Third Edition). Academic Press, San Diego, pp. 191-248.
Caridad Cancela, R., de Abreu, C. A., Paz-Gonzalez, A. (2002): DTPA and Mehlich-3 micronutrient extractability in natural soils. *Commun. Soil Sci. Plant Anal.* 33, 2879-2893.
Chaignon, V., Sanchez-Neira, I., Herrmann, P., Jaillard, B., Hinsinger, P. (2003): Copper bioavailability and extractability as related to chemical properties of contaminated soils from a vine-growing area. *Environ. Pollut.* 123(2), 229-238.
Chen, B., Shan, X. Q., Qian, J. (1996): Bioavailability index for quantitative evaluation of plant availability of extractable soil trace elements. *Plant Soil* 186(2), 275-283.
da Fonseca, A. F., Caires, E. F., Barth, G. (2010): Extraction methods and availability of micronutrients for wheat under a no-till system with a surface application of lime. *Scientia Agricola* 67(1), 60-70.
Elrashidi, M. A., Mays, M. D., Lee, C. W. (2003): Assessment of Mehlich3 and ammonium bicarbonate-DTPA extraction for simultaneous measurement of fifteen elements in soils. *Commun. Soil Sci. Plant Anal.* 34(19-20), 2817-2838.
Fageria, N. K., Baligar, C., Clark, R. B. (2002): Micronutrients in crop production, in Advances in Agronomy (Vol. Volume 77). Academic Presspp. 185-268.
FAO. (2018). FAOSTAT: Crops. Retrieved from http://www.fao.org/faostat/en/#data/QC
Gediga, K., Spiak, Z., Piszcz, U., Bielecki, K. (2015): Suitability of Different Soil Extractants for Determination of Available Cu and Mn Contents in Polish Soils. *Commun. Soil Sci. Plant Anal.* 46, 81-93.
Gupta, S. K., Aten, C. (1993): Comparison and evaluation of extraction media and their suitability in a simple model to predict the biological relevance of heavy metal concentrations in contaminated soils. *Int. J. Environ. Anal. Chem.* 51(1), 25 - 46.
Hedley, M. J. (2008): Techniques for assessing nutrient bioavailability in soils: current and future issues, in Naidu, R. (ed.): Developments in Soil Science, Vol 32. Elsevier B. V.pp. 283-327.
Hosseinpur, A. R., Zarenia, M. (2012): Evaluating chemical extractants to estimate available potassium for pinto beans (*Phaseolus vulgaris*) in some calcareous soils. *Plant Soil Environ.* 58(1), 42-48.
Houba, V. J. G., Temminghoff, E. J. M., Gaikhorst, G. A., van Vark, W. (2000): Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. *Commun. Soil Sci. Plant Anal.* 31(9-10), 1299-1396.
Joshi, D., Srivastava, P. C., Dwivedi, R., Pachauri, S. P. (2014): Chemical speciation of Zn in acidic soils: suitable soil extractant for assessing Zn availability to maize (*Zea mays* L.). *Chem. Speciat. Bioavailab.* 26(3), 148-157.
Joshi, D., Srivastava, P. C., Dwivedi, R., Pachauri, S. P., Shukla, A. K. (2017): Chemical Fractions of Mn in Acidic Soils and Selection of Suitable Soil Extractants for Assessing Mn Availability to Maize (*Zea mays* L.). *Commun. Soil Sci. Plant Anal.* 48(8), 886-897.
Kamprath, E. J., Smyth, T. J. (2005): Liming, in Hillel, D. (ed.): Encyclopedia of Soils in the Environment. Elsevier, Oxford, pp. 350-358.
Katyal, J. C., Sharma, B. D. (1991): DTPA-extractable and total Zn, Cu, Mn, and Fe in Indian soils and their association with some soil properties. *Geoderma* 49(1-2), 165-179.
Kirkby, E. (2012): Chapter 1 - Introduction, Definition and Classification of Nutrients, in Marschner, P. (ed.): Marschner's Mineral Nutrition of Higher Plants (Third Edition). Academic Press, San Diego, pp. 3-5.
Korzeniowska, J., Stanislawska-Glubiak, E. (2015): Comparison of 1 M HCl and Mehlich 3 for Assessment of the Micronutrient Status of Polish Soils in the Context of Winter Wheat Nutritional Demands. *Commun. Soil Sci. Plant Anal.* 46(10), 1263-1277.
Lake, D. L., Kirk, P. W. W., Lester, J. N. (1984): Fractionation, characterization, and speciation of heavy-metals in sewage-sludge and sludge-amended soils - a review. *J. Environ. Qual.* 13(2), 175-183.
Lindsay, W. L., Cox, F. R. (1985): Micronutrient soil testing for the tropics, in Vlek, P. L. G. (ed.): Micronutrients in Tropical Food Crop Production. Developments in Plant and Soil Sciences, vol 14. Springer, Dordrecht, pp. 169-200.
Lindsay, W. L., Norvell, W. A. (1978): Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Am. J.* 42(3), 421-428.
Lollato, R. P., Ochsner, T. E., Arnall, D. B., Griffin, T. W., Edwards, J. T. (2019): From Field Experiments to Regional Forecasts: Upscaling Wheat Grain and Forage Yield Response to Acidic Soils. *Agron. J.* 111(1), 287-302.
Lucena, J. J., Bascones, I. (1993): AB-DTPA cation extraction in Spanish soil samples. *Commun. Soil Sci. Plant*

Anal. 24(17-18), 2427-2440.

Madurapperuma, W. S., Kumaragamage, D. (2008): Evaluation of ammonium bicarbonate–diethylene triamine penta acetic acid as a multinutrient extractant for acidic lowland rice soils. *Commun. Soil Sci. Plant Anal.* 39(11-12), 1773-1790.

Malathi, P., Stalin, P. (2018): Evaluation of AB - DTPA Extractant for Multinutrients Extraction in Soils. *International Journal of Current Microbiology and Applied Sciences* 7(3), 1192-1205.

McBride, M. B., Nibarger, E. A., Richards, B. K., Steenhuis, T. (2003): Trace metal accumulation by red clover grown on sewage sludge-amended soils and correlation to Mehlich 3 and calcium chloride-extractable metals. *Soil Sci.* 168(1), 29-38.

McLaughlin, M. J., Zarcinas, B. A., Stevens, D. P., Cook, N. (2000): Soil testing for heavy metals. *Commun. Soil Sci. Plant Anal.* 31, 1661-1700.

Meers, E., Samson, R., Tack, F. M. G., Ruttens, A., Vandegehuchte, M., Vangronsveld, J., Verloo, M. G. (2007): Phytoavailability assessment of heavy metals in soils by single extractions and accumulation by Phaseolus vulgaris. *Environ. Exp. Bot.* 60(3), 385-396.

Mehlich, A. (1984): Mehlich-3 soil test extractant - a modification of Mehlich-2 extractant. *Commun. Soil Sci. Plant Anal.* 15(12), 1409-1416.

Menzies, N. W., Donn, M. J., Kopittke, P. M. (2007): Evaluation of extractants for estimation of the phytoavailable trace metals in soils. *Environ. Pollut.* 145(1), 121-130.

Monterroso, C., Álvarez, E., Marcos, M. L. F. (1999): Evaluation of Mehlich 3 reagent as a multielement extractant in mine soils. *Land Degrad. Dev.* 10(1), 35-47.

O'Connor, G. A. (1988): Use and misuse of the DTPA soil test. *J. Environ. Qual.* 17(4), 715-718.

Obrador, A., Álvarez, J. M., López-Valdivia, L. M., González, D., Novillo, J., Rico, M. I. (2007): Relationships of soil properties with Mn and Zn distribution in acidic soils and their uptake by a barley crop. *Geoderma* 137(3-4), 432-443.

Olsen, S. R., Cole, C. V., Watanabe, F. S., Dean, L. A. (1954): Estimation of available phosphorous in soils by extraction with sodium bicarbonate. USDA, Government Printing Office, Washington, DC.

Page, A. L., Miller, R. H., Keeney, D. R. (1982): Methods of soil analyses. Part II. Chemical and microbiological properties (Vol. 9). American Society of Agronomy, Madison, WI, USA.

Panda, R., Patra, S. K. (2018): Assessment of Suitable Extractants for Predicting Plant-available Potassium in Indian Coastal Soils. *Commun. Soil Sci. Plant Anal.* 49(10), 1157-1167.

Pettigrew, W. T. (2008): Potassium influences on yield and quality production for maize, wheat, soybean and cotton. *Physiol. Plant* 133(4), 670-681.

Pickering, W. F. (1986): Metal ion speciation -- soils and sediments (a review). *Ore Geology Reviews* 1(1), 83-146.

Pueyo, M., López-Sánchez, J. F., Rauret, G. (2004): Assessment of CaCl2, NaNO3 and NH4NO3 extraction procedures for the study of Cd, Cu, Pb and Zn extractability in contaminated soils. *Anal. Chim. Acta* 504(2), 217-226.

Rodríguez-Suárez, J. A., Arias, M., López, E., Soto, B. (2008): Comparison of multi-element to single-element extractants for macro- and micronutrients in acid soils from Spain. *Commun. Soil Sci. Plant Anal.* 39(1-2), 231-240.

Sauve, S., Norvell, W. A., McBride, M., Hendershot, W. (2000): Speciation and complexation of cadmium in extracted soil solutions. *Environ. Sci. Technol.* 34(2), 291-296.

Schwertmann, U., Taylor, R. M. (1989): Iron oxides. Minerals in soil environments (2nd ed.). Soil Science Society of America Madison, USA.

Seth, A., Sarkar, D., Datta, A., Mandal, B., Chowdhury, A., Masto, R. E., Chakravarty, A. K., Hazra, G. C., Badole, S., Batyabal, K., Saha, S. (2017): Suitability of Complex Extractants for Assessment of Available Soil Zinc for Nutrition of Rice (*Oryza sativa* L.) in Subtropical India. *Soil Sci.* 182(1), 28-35.

Seth, A., Sarkar, D., Masto, R. E., Batabyal, K., Saha, S., Murmu, S., Das, R., Padhan, D., Mandal, B. (2018): Critical limits of Mehlich 3 extractable phosphorous, potassium, sulfur, boron and zinc in soils for nutrition of rice (*Oryza sativa* L.). *J. Soil Sci. Plant Nutr.* 18(2), 512-523.

Sharma, S. K., Sharma, A., Rana, S., Kumar, N. (2018): Evaluation of multi-nutrient extractants for determination of available P, K, and micronutrient cations in soil. *J. Plant Nutr.* 41(6), 782-792.

Shuman, L. M. (1991): Chemical forms of micronutrients in soil, in Morvedt, J. J., Cox, F. R., Shuman, L. M., Welch, R. M. (eds.): Micronutrients in Agriculture (2nd ed.). SSSA, Madison, WI, pp. 113-144.

Sims, J. T., Johnson, G. V. (1991): Micronutrient soil tests, in Mortvedt, J. J. (ed.): Micronutrients in agriculture. SSSA pp. 427-476.

Singh, B., Natesan, S. K. A., Singh, B. K., Usha, K. (2005): Improving zinc efficiency of cereals under zinc deficiency. *Current Science* 88(1), 36-44.

Soltanpour, P. N. (1985): Use of ammonium bicarbonate DTPA soil test to evaluate elemental availability and

toxicity. *Commun. Soil Sci. Plant Anal.* 16(3), 323-338.
Soltanpour, P. N., Schwab, A. P. (1977): New soil test for simultaneous extraction of macro-nutrients and micro-nutrients in alkaline soils. *Commun. Soil Sci. Plant Anal.* 8(3), 195-207.
Soriano-Disla, J. M., Gómez, I., Navarro-Pedreno, J., Lag-Brotons, A. (2010): Evaluation of single chemical extractants for the prediction of heavy metal uptake by barley in soils amended with polluted sewage sludge. *Plant Soil* 327(1-2), 303-314.
Viets, F. G. (1962): Micronutrient availability - chemistry and availability of micronutrients in soils. *J. Agric. Food Chem.* 10(3), 174-178.
von Uexküll, H. R., Mutert, E. (1995): Global extent, development and economic impact of acid soils. *Plant Soil* 171(1), 1-15.
Wang, J. J., Harrell, D. L., Henderson, R. E., Bell, P. F. (2004): Comparison of soil-test extractants for phosphorus, potassium, calcium, magnesium, sodium, zinc, copper, manganese, and iron in Louisiana soils. *Commun. Soil Sci. Plant Anal.* 35(1-2), 145-160.
Zhang, C., Niu, D., Ren, Y., Fu, H. (2018): Extractability of nutrients using Mehlich 3 and ammonium bicarbonate-DTPA methods for selected grassland soils of China. *Plant Soil Environ.* 64(9), 448-454.
Zhu, Q., Ozores-Hampton, M., Li, Y. C., Mylavarapu, R. S. (2017): Comparing Extractants for Calibrating Potassium Rates for Tomato Grown on a Calcareous Soil. *Soil Sci. Soc. Am. J.* 81(6), 1621-1628.
Ziadi, N., Sen-Tran, T. (2007): Mehlich 3-Extractable elements, in Carter, M. R., Gregorich, E. G. (eds.): Soil Sampling and Methods of Analysis (2nd Ed ed.). CRC Press, Boca Raton, FL, pp. 81-88.